ENZYMATIC STUDIES OF LEUCEMIC MOUSE LIVER

BY

RICHARD ELMORE MAXWELL

B. S., Southern Methodist University, 1943

AN ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN CHEMISTRY
IN THE GRADUATE SCHOOL OF THE
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I. GENERAL INTRODUCTION

Leucemia is a disease most often characterized by an enormous increase in the number of white blood cells. Highly inbred strains of mice have been developed which spontaneously acquire leucemia Leucocytes from a diseased mouse, injected into a normal mouse of the same strain, give rise to a virulent form of the disease.[1] This indicates that the leucocytes possess the ability to proliferate in an uncontrolled manner, and that leucemia is a form of cancer.

These cancerous leucocytes infiltrate into the tissues of nearly all of the organs of the body. A study of the effect of this malignant invasion on the enzymatic activity of the affected organs may contribute to an understanding of the metabolic peculiarities of neoplastic growth.

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II. ANAEROBIC GLYCOLYSIS, CYTOCHROME OXIDASE, AND SUCCINIC OXIDASE ACTIVITIES OF LEUCEMIC MOUSE LIVER

Introduction

An increase in the rate of anaerobic glycolysis is an almost invariable characteristic of neoplastic tissues. It has been shown that the liver anaerobic glycolysis rate of the Rf and Ak strains of leucemic mice is correlated with the extent of infiltration by malignant leucocytes.[1] It was desired to confirm this relation in the mouse strain used in the present investigation.

Cytochrome oxidase and succinic oxidase activities are

greatly reduced in neoplastic tissues.[2] There has been no previous study of the activities of these enzymes in organs infiltrated by malignant leucocytes.

Experimental

The livers of leucemic mice of the C-58* strain were used in this study. The degree of infiltration by leucocytes was histologically estimated in the case of each liver.

For the anaerobic glycosis determinations, liver slices were suspended in a bicarbonate buffer medium containing glucose as the substrate. The gas atmosphere was a mixture of 95% nitrogen and 5% carbon dioxide. Carbon dioxide production was measured in a conventional Warburg apparatus at 38°C. The results showed that the rate of anaerobic glycolysis was roughly proportional to the degree of infiltration. The $Q_A^{N_2}$ values (microliters of carbon dioxide produced per hour per milligram of dry tissue) ranged from 1.1 at an estimated infiltration of less than 1% to 6.2 at an infiltration of 33%.

Liver homogenates were used for the determinations of cytochrome oxidase and succinic oxidase activities. Oxygen uptake was measured in the Warburg apparatus at 38°C. An excess of cytochrome *c* was added to both systems, and ascorbate was used to reduce the cytochrome in the cytochrome oxidase assay.[3] The results showed that succinic oxidase activity is lowered by malignant infiltration; QO_2 values (microliters of oxygen uptake per hour per milligram of dry tissue) ranged from 89 ± 20 for nine livers with less than 2% infiltration down to 51 ± 22 for five livers with more than 6% infiltration. Cytochrome oxidase activity, however, was not significantly related to the degree of infiltration. Thus, six livers with infiltrations of less than

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2% had QO_2 values of 115 ± 18, and five livers with more than 6% infiltration had QO_2 values of 108 ± 20.

Summary

In the leucocyte-infiltrated livers of leucemic C-58 mice, anaerobic glycolysis activity is increased, succinic oxidase activity is decreased, and cytochrome oxidase activity is unaffected.[4,5]

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III. THE SIGNIFICANCE OF SUCCINIC OXIDASE ACTIVITY IN THE PRESENCE AND ABSENCE OF ADDED CYTOCHROME *C*

Introduction

Schneider and Potter[1] observed that succinic oxidase activity of rat liver homogenates, in the absence of added cytochrome *c*, decreased with decreasing tonicity of the medium in which the homogenization was performed. These authors believed that homogenization in a hypotonic medium favored the rupture of cells, and that succinic oxidase activity

in the absence of added cytochrome *c* was due only to whole cells.

However, Elliott and Greig[2] had previously shown that homogenates prepared in hypertonic buffer had high succinic oxidase activity which was little increased by adding cytochrome *c*, and a very low cytochrome oxidase activity which was greatly increased by adding cytochrome *c*. Since whole cells are impermeable to cytochrome *c*, there were probably few whole cells present, although the high succinic oxidase activity in the absence of added cytochrome *c* would indicate, according to Schneider and Potter, almost no broken cells.

The data obtained in this study substantiate the belief that isotonic homogenates contain few whole cells.

Experimental

Mouse liver homogenates were prepared in either hypotonic or isotonic buffers. Succinic oxidase activity was determined in the presence and absence of added cytochrome *c*, and cytochrome oxidase activity was determined in the presence of added cytochrome *c*. The cytochrome oxidase activity was practically the same in both types of homogenates, and therefore the degree of cell rupture was the same. However, succinic oxidase activity was greatly decreased in the hypotonic homogenates. These results indicate that homogenization in a hypotonic medium must have an effect on subcellular organization, possibly by removing the physiologically present cytochrome *c* from the enzyme surface. The enzyme itself is apparently not affected by this treatment, since addition of cytochrome *c* restored full activity.

Summary

In the absence of added cytochrome *c*, the succinic oxidase activity of mouse liver homogenates prepared in hypotonic buffer was lower than that of homogenates prepared in isotonic buffer. The lowered activity may be best explained as an effect of the hypotonic homogenization on subcellular

organization.

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IV. CYTOCHROME *C* PREPARED FROM HORSE HEART POWDER

Introduction

In connection with these studies, it became of interest to investigate possible commercial sources of cytochrome *c*. The Viobin Corporation, Monticello, Illinois, is engaged in the development of a process for the preparation of cytochrome *c* from dehydrated, defatted horse heart powder. Experimental samples were examined in this laboratory for their purity and activity. Cytochrome prepared by the Keilin and Hartree procedure[1] was similarly tested for comparison.

Experimental

A considerable quantity of cytochrome precipitated from many of the commercial samples on extensive dialysis against water. Boiling of the solutions also resulted in the coagulation of part of the cytochrome present. Boiling or dialysis did not affect solutions of Keilin and Hartree cytochrome. These results indicate that some of the commercial preparations contained denatured cytochrome.

The purity of the solutions was estimated from the iron content and from absorption characteristics. These results showed that the most active commercial preparation was about 75% pure. However, the Keilin and Hartree prepara-

tions also varied in purity, four samples ranging from about 75% to 95% pure.

The catalytic potency of the samples was tested in cytochrome oxidase and succinic oxidase assays of rat liver homogenates. The commercial samples were usually somewhat less active than Keilin and Hartree cytochrome. The cytochrome remaining in solution after boiling was more active, but in most cases did not attain the activity of the Keilin and Hartree product.

Summary

Experimental samples of cytochrome *c* prepared from horse heart powder were found to vary in activity, possibly because of the presence of denatured material.

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VITA

The author was born on August 8, 1921, in Dallas, Texas. He attended the elementary and secondary schools of Highland Park, and entered Southern Methodist University in the fall of 1939. The Bachelor of Science degree was received in June of 1943. From June, 1943, to October, 1944, he was employed as a Research Assistant by the Magnolia Petroleum Company in Dallas. In October of 1944 he entered the Graduate School of the University of Illinois as a teaching Assistant, and held this position until June, 1947. During the summer session of 1947, he held a Graduate School Fellowship.